LightMix® Kit *Adenovirus*

Cat.-No. 40-0303-16

Kit with reagents for the quantitative detection of *Adenovirus* DNA using the LightCycler® 1.x / 2.0 Instruments.

Lyophilized mix of primers and probes (6 tubes with 16 rxns each) for a total of 96 reactions with a final volume of 20 µl each - **store protected from light at room temperature (18-25°C), do NOT freeze!**

Additional reagents required (Roche Diagnostics):

LightCycler® FastStart DNA Master^{PLUS} HybProbe	Cat.-No. 03 515 575 001
or LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
LightCycler® Color Compensation Set (LightCycler® 1.x Instrument)	Cat.-No. 12 158 850 001
or LightCycler® Multicolor Demo Set	Cat.-No. 03 624 854 001
High Pure PCR Template Preparation Kit	Cat.-No. 11 796 828 001

1. Introduction

Adenoviruses infect the membranes of the respiratory tract, eyes, intestines, and urinary tract.

They account for about 10% of acute respiratory infections in children and are a frequent cause of diarrhea.
A total of 51 serotypes of human adenovirus (HAdV) have been described which are divided into six groups named from A to F.

Group	Type
A	12, 18, 31
B	3, 7, 11, 14, 21, 34, 35
C	1, 2, 5, 6
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39
E	4
F	40, 41

The LightMix® Kit for the detection of *Adenovirus* DNA provides a fast, easy and accurate system to identify and quantify this target in a nucleic acid extract. A control amplification reaction acts as internal positive control (IC).

This LightMix® Kit tested with the Roche Diagnostics "LightCycler® FastStart^{PLUS} DNA Master Hybridization Probes" ready–to-use reaction mix in the LightCycler® Instrument 2.0.

2. Description

A 129 bp fragment of the *Adenovirus* genome (Hexon) is amplified with specific primers and detected with probes labeled with LightCycler® Red 640 (detected in channel 640). The PCR products are verified by running a melting curve, but the results are not useful for assigning which virus group the samples belong to. For the identification of a virus group from the PCR products generated with this LightMix® kit a Low-density array can be used and is available at CHIPRON GmbH.

A PCR product of 278 bp is formed from the internal positive control DNA. The IC can be used as an external positive control (reaction in a separate capillary) to increase the sensitivity for the detection of the *Adenoviruses*. This is due to the fact that the detection of *Adenoviruses* is a multi primer system. The probes are labeled with the LightCycler® Red 705 (detected in channel 705) and have a specific Tm of about 67-69°C. The IC is supplied separately to allow running the assay with or without IC.

The use of a color compensation file generated with the LightCycler®-Color Compensation Kit or with the LightCycler® Multicolor Demo Set is a prerequisite to run the internal control.

The supplied standard row (based on adenovirus group B) allows the absolute quantification of the unknown samples.

For use in LightCycler® Instruments other than 2.0 use channel F2 instead of channel 640 and channel F3 instead of channel 705 for detection.

3. Set contents

6 Vials with blue clip containing premixed lyophilized primers and hybridization probes for 16 reactions each
6 Vials with white clip containing the internal positive control (IC)
1 Row with 6 lyophilized standards from 10^1 to 10^6 target equivalents per reaction of *Adenovirus* DNA
1 Sealing foil for the standard row

4. Programming

The protocol consists of four program steps

- 1: Denaturation: samples denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting				Cooling
Parameter									
Analysis Mode	None	Quantification mode			Melting Curves mode				None
Cycles	1	50			1				1
Target [℃]	95	95	62	72	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	00:00:15	00:00:05	00:00:20	00:00:30	00:00:00	00:00:30
Ramp Rate [℃/s]	20	20	20	20	20	20	20	0.2	20
Sec Target [℃]	-	-	55	-	-	-	-	-	-
Step Size [℃]	-	-	0.5	-	-	-	-	-	-
Step Delay (Cycles)	-	-	1	-	-	-	-	-	-
Acquisition Mode	None	None	Single	None	None	None	None	Cont	None

(Melting not relevant for detection)

5. Data analysis

Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual of the LightCycler® – Color Compensation Kit.

Perform data analysis, as described in the LightCycler® operator's manual.

View *Adenovirus* data in channel 640, Quantification mode. The negative control (NTC) should show no signal. For the identification of the PCR product view *Adenovirus* data in channel 640, Melting Curves mode.

If the internal positive control is used, view *Adenovirus* data in channel 640, Quantification mode and the IC in channel 705, quantification mode. The negative control and the low-concentrated *Adenovirus* DNA samples (10 to 1,000 copies) should show an amplification curve for the IC with a CP approximately at cycle 27.

Typical results (Software Version 4.0)
The provided standard row of cloned and purified DNA with concentrations in the range from 10^6 copies/rxn to 10^1 copies/rxn should have CPs between cycles 18 and 35 (CPs calculated with Second Derivative Maximum method).

6. Product characteristics

PCR results are obtained within 1 hour.

Sensitivity
These reagents detect 100 copies of *Adenovirus* DNA (in an exemplary system, using cloned targets as reference). A detection limit of 10 copies of *Adenovirus* DNA is possible by running the assay without IC.

Measuring range
The linear measuring range of the assay is 10^2 to 10^6 copies of *Adenovirus* DNA.

Storage and Stability
- Lyophilized reagents are stable for at least 3 months after shipment if stored protected from light at room temperature (18-25℃).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days if stored protected from light and refrigerated (4℃).

7. Experimental protocol

The following procedure was developed for use with the LightCycler® Instrument 1.x / 2.0.
Start programming before preparing the solutions. See the LightCycler® operator's manual for details.

Sample material: Use aqueous nucleic acid preparations (e.g. High Pure PCR Template Preparation Kit).

Negative control: Always run at least one negative control - replace the template DNA with water.

Positive control: Run a positive control - replace the template DNA with the provided control DNA.
For quantification always use a fresh solution prepared from the provided standard row (single use only).

7.1 Preparation of parameter-specific reagents and reagents for the IC (16 reactions):

One reagent vial with a **blue** clip contains all primers and probes to run 16 LightCycler® reactions for *Adenovirus*.
One reagent vial with a **white** clip contains all primers, probes and DNA to run 16 LightCycler® reactions for the IC.

Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

►Use 4 µl **reagent** for a 20 µl PCR reaction.

This solution is stable for three days or longer if stored refrigerated at 4°C. Avoid prolonged exposu re to light.

7.2 Preparation of the standard row (quantification)

The target DNA is provided in 6 different quantities to yield from 10^1 to 10^6 target molecules in 5 µl once resuspended. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. Add 40 µl PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution up and down 10 times.

►Use 5 µl standard for a 20 µl PCR reaction

This standard solution is not long-term stable and will loose sensitivity under prolonged storage. Use only fresh prepared solutions as quantification references. Older solutions can be used as a qualitative standard (positive control).
After adding the target DNA to the LightCycler® reaction mix use the provided sealing foil to close the vials in order to avoid changes in the concentration due to evaporation.
Please note that opening of these vials may cause contaminations of the work-space (aerosol).

7.3 Preparation of the LightCycler® reaction mix

In a reaction tube cooled below 4°C, prepare the re action mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart^{PLUS} kit		**For use with the Roche FastStart kit**
Single reaction	**Component**	**Single reaction**
3.0 µl	water, PCR-grade (colorless cap, provided with the Roche FastStart or FastStart^{PLUS} kit)	3.4 µl
--	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)	1.6 µl
4.0 µl	**reagent** mix (parameter specific reagents containing primers and probes, see **7.1**)	4.0 µl
4.0 µl	**IC** mix – **OPTIONAL** - (IC reagents containing primers, probes and DNA, see **7.1**)	4.0 µl
4.0 µl	FastStart mix (vial 1 (red cap), combined from vials 1a and 1b, see Roche manual)	2.0 µl
15.0 µl	**Volume of reaction mix**	**15.0 µl**

To include the internal positive control add 4 µl of the IC reagent per reaction to the reaction mix.
To run the assay without the internal control add additional 4 µl PCR-grade water instead of the IC reagent to the reaction mix.

Mix gently, spin down and transfer 15µl each of the reaction mix to a LightCycler® capillary.
Add 5 µl of sample or standard (standard dilutions of control target, see **7.2**) to each capillary to give a final reaction volume of **20 µl.**

Start run.

8. Sample data - typical results

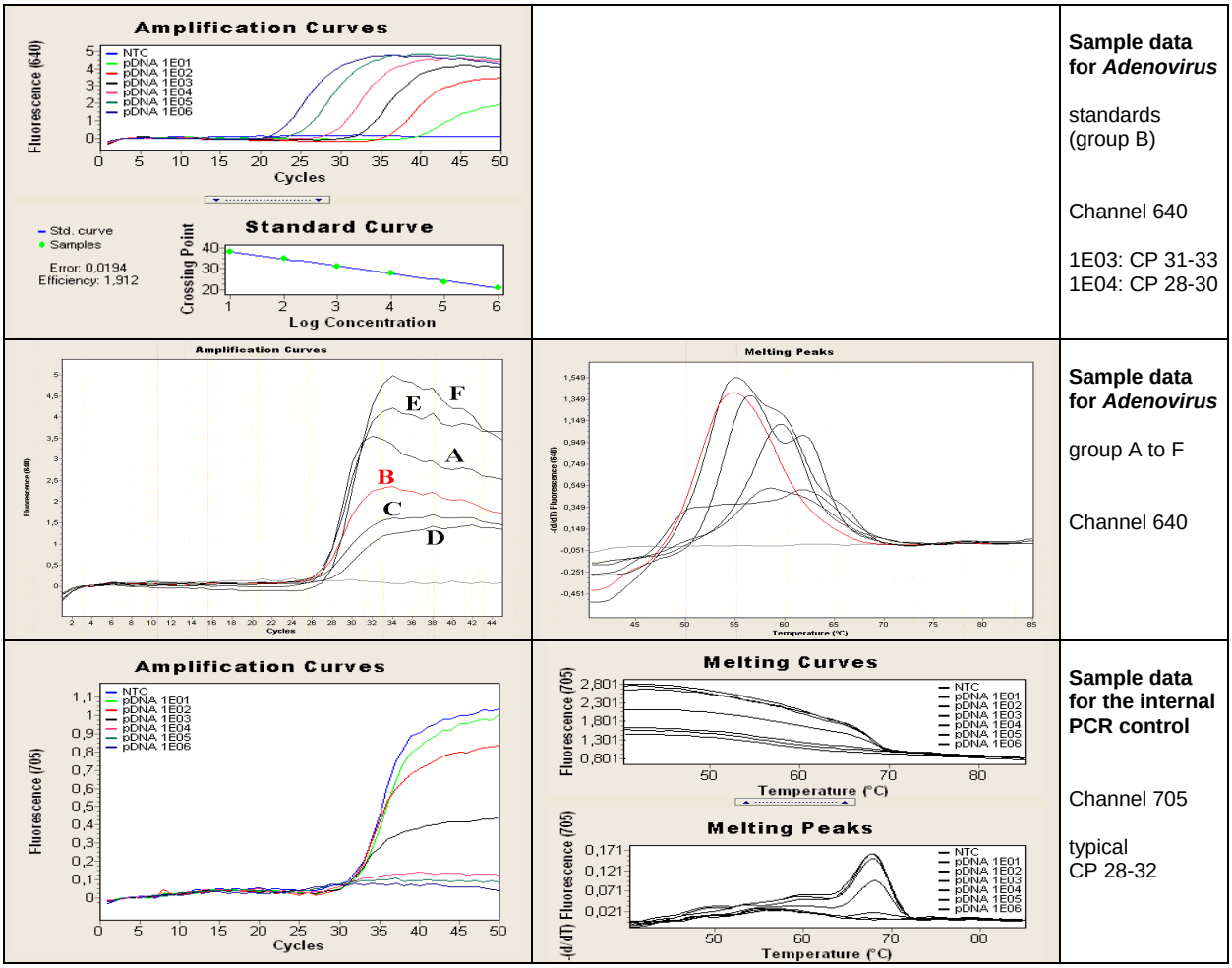

Fig.1. Sample data for the *Adenovirus* detection system.

Upper panels: Data from channel 640. Left panel quantification (Second Derivative Maximum) with calibration curve.
Middle panels: Data from channel 640. Left panel quantification (Second Derivative Maximum), right panel melting analysis for the different groups of *Adenovirus* (not relevant for detection, shape varies with concentration).
Lower panels: Data from channel 705. Left panel quantification mode, right panel melting analysis for the IC (not relevant for detection).

9. Interpretation of data

Adenovirus (sample)	IC (sample)	NTC	Result
no amplification	detectable	negative	Negative
amplification signal	not relevant	negative	Positive
no amplification	not detectable	not relevant	PCR failure, repeat experiment
amplification signal	not relevant	positive	Contamination, repeat experiment

Tab. 3. Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)

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